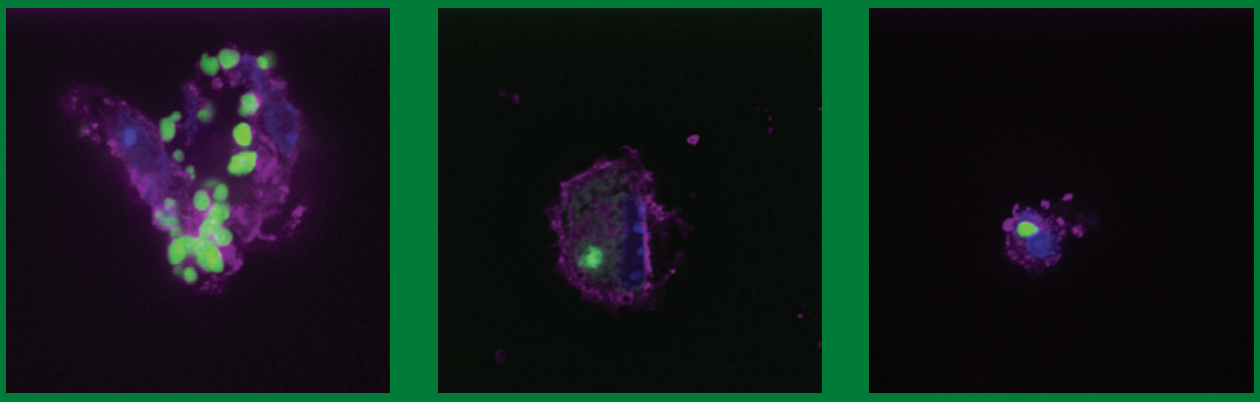

**Research has revealed a link between obesity and the inflammatory response of the immune system. As the body takes in excess calories, fat cells increase in size to store the extra fat. Eventually the fat cells become overloaded and begin to release molecules that attract inflammatory cells, specifically macrophages. When macrophages are continually recruited to the fat cells, as is the case in obesity, a chronic state of inflammation can occur, contributing to insulin resistance and type 2 diabetes.**

**NIDDK-supported scientists recently sought to apply "RNA interference" to modulate the functioning of macrophages, so as to dampen inflammation. RNA interference, discovered previously by other researchers, involves reducing the levels of specific proteins with molecules called small interfering RNAs or siRNAs. This revolutionary technique has enormous therapeutic potential, but its application has been limited by lack of an effective and safe oral delivery vehicle. In a novel approach toward overcoming this obstacle, NIDDK-supported scientists developed a delivery vehicle called glucan-encapsulated siRNA particles, or GeRPs. When GeRPs (shown in green) were fed to mice, they were taken up by macrophages (shown in magenta) and migrated to several different tissues. These images illustrate the presence of GeRPs in mouse spleen (left), liver (middle), and lung (right) tissues. By feeding mice GeRPs with siRNA to a specific inflammatory protein, the scientists were able to reduce levels of this protein and to suppress inflammation in mice. Because macrophages and inflammation are involved in many diseases, developing a strategy for therapy in the macrophages might be applied to many other diseases. As described in this chapter and elsewhere in this book, NIDDK-supported research is making advances in understanding and treating numerous NIDDK-related diseases.**

*Image provided by Dr. Michael P. Czech and reprinted by permission from Macmillan Publishers Ltd: Nature 458: 1180-1184, copyright 2009.*

# Cross-Cutting Science

*Advances in medicine are largely dependent upon the accumulation of new knowledge about biologic processes, often at the smallest levels of an organism—its genes, the proteins they encode, the inner workings of cells, and the ways cells communicate with each other. Major strides in fighting disease can be traced back to laboratory studies whose immediate relevance to health could not have been fully known or appreciated at the time they were conducted. Opportunities to make exciting new discoveries are arising ever more rapidly with the development of new technologies, new approaches, and even new scientific disciplines as teams of talented, creative researchers join together to pursue increasingly complex challenges. Described in this chapter are several recent studies, each of which spans multiple areas within the NIDDK research mission. The insights gained through this kind of research can be expected to aid progress in many scientific endeavors, for today's research advances may lead to tomorrow's cures.*

## NIDDK CELEBRATES ITS 60TH ANNIVERSARY

In 2010, the NIDDK celebrates 60 years since its founding. Over the course of its history, the Institute that is known today as the National Institute of Diabetes and Digestive and Kidney Diseases is proud to have supported and conducted research on many of the most serious diseases affecting public health. Affecting people of all ages and ethnic groups, the diseases within the NIDDK research mission encompass some of the most common, severe, and disabling conditions, as well as less prevalent but nonetheless debilitating diseases, affecting Americans today: endocrine and metabolic diseases and disorders such as diabetes and obesity, digestive diseases such as hepatitis and inflammatory bowel disease, kidney and urologic diseases such as kidney failure and prostate enlargement, and blood diseases such as the anemias.

The research advances made possible through 60 years of NIDDK support have saved lives, improved quality of life, and laid the foundation for future progress. The Institute has supported a number of winners of the world's greatest scientific honor. Many have won the Nobel Prize in Physiology or Medicine, and others have received the Nobel Prize in Chemistry. These include extramural scientists at universities and other research institutions across the country who have been supported by the NIDDK (Institute grantees), as well as scientists within the Institute's Division of Intramural Research.

As part of a year-long celebration to mark its 60th anniversary, the Institute has planned a variety of activities. Scientific symposia are planned for the annual meetings of several professional societies and organizations with a focus on diseases within the Institute's mission. The NIDDK is also publishing a booklet for the public that commemorates its 6 decades of support for biomedical research and highlights discoveries that have been made by NIDDK-funded investigators and NIDDK intramural scientists. The anniversary celebrations will additionally feature an NIDDK Anniversary Scientific Symposium on the National Institutes of Health (NIH) campus in September 2010.

*History of the NIDDK:* On August 15, 1950, President Harry S. Truman signed into law the Omnibus Medical Research Act, establishing the National Institute of Arthritis and Metabolic Diseases (NIAMD)—which would become today's NIDDK. The new Institute incorporated the laboratories of the Experimental Biology and Medicine Institute and expanded to include clinical investigation in rheumatic diseases, diabetes, and a number of metabolic, endocrine, and gastrointestinal diseases. That same year, the NIAMD Advisory Council held its first meeting and recommended approval of NIAMD's first grants. In November 1950, U.S. Surgeon General Leonard Scheele formally established NIAMD.

Over the years, the NIAMD evolved into the National Institute of Arthritis, Metabolism, and Digestive Diseases (in 1972) and the National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases (in 1981). In 1986, the Institute's Division of Arthritis, Musculoskeletal and Skin Diseases became the core of a new, independent Institute. The NIDDK then acquired its current name, the National Institute of Diabetes and Digestive and Kidney Diseases.

## AMERICAN RECOVERY AND REINVESTMENT ACT OF 2009 AND NIH BIOMEDICAL RESEARCH

The American Recovery and Reinvestment Act of 2009 (also referred to as ARRA or the Recovery Act) was signed into law by President Barack Obama on February 17, 2009. The legislation provides a total of $10.4 billion to the NIH: $8.2 billion in extramural funding for scientific research; $1 billion to the National Center for Research Resources (NCRR) to support extramural construction, repairs, and alterations in support of all research institutions that receive NIH funding; $300 million for shared instrumentation and other capital equipment to support all NIH activities; $500 million for high priority repair, construction, and improvement projects on NIH campuses; and $400 million to support comparative effectiveness research. The NIDDK received $445 million of these funds, as well as additional funds from the Office of the NIH Director and NIH Common Fund allocations.

The NIDDK is deploying funds made available through the Recovery Act to support highly meritorious applications for research projects, to target supplements to accelerate the pace of ongoing science, and to fund new NIH activities, such as Challenge Grants. The Institute is working closely with the broader NIH community and the U.S. Department of Health and Human Services to ensure that scientific merit, as well as process transparency and accountability, are the guiding principles behind the Institute's implementation of the Recovery Act.

- For an overview of the Recovery Act, visit: *www.recovery.gov/*
- For more information about the Recovery Act at NIH, visit: *www.nih.gov/recovery*
- For more information on NIDDK's implementation of the Recovery Act, see *www2.niddk.nih.gov/Recovery/*

## RECENT GENETICS STUDIES: PAVING A WAY TOWARD IMPROVING PEOPLE'S HEALTH

New research resources, including the wealth of information from the Human Genome Project and the International HapMap project, are making it easier for scientists to identify genes that influence a person's likelihood of developing a variety of genetically complex diseases. Ranging from rare conditions to very common diseases such as type 2 diabetes, these diseases result from variations in multiple genes. Because these variations may individually have only modest contributions to disease susceptibility—along with other genetic and environmental factors—it has been challenging to identify the disease-associated genes. Taking advantage of the new research tools and technologies, scientists are conducting genome-wide association studies to identify genetic differences between people with specific illnesses or conditions, and healthy individuals. Through this comparison, it has become possible to identify genetic differences that may affect whether an individual develops a particular disease.

Recent genome-wide association studies and other genetics studies have led to an explosion in the identification of genes and gene regions important in diseases within the NIDDK mission. Often the associated gene is unexpected—the function of the gene may be completely unknown or it may be involved in cellular processes that were not thought to be important in the particular disease. Some examples are highlighted in the chapters of this compendium, including genetic variants associated with type 1 diabetes, ulcerative colitis, gout, primary biliary cirrhosis, and hepatitis C.

Using genome-wide association studies, over 40 different regions of the genome have been identified that influence a person's risk of developing type 1 diabetes. Type 1 diabetes is an autoimmune disease in which the immune system launches a misguided attack and destroys the insulin-producing beta cells of the

pancreas. More information on type 1 diabetes and this study can be found in the Diabetes, Endocrinology, and Metabolic Diseases chapter.

Ulcerative colitis (UC) is a form of inflammatory bowel disease that causes inflammation in the tissues lining the colon and rectum. To expand knowledge of genetic contributors to UC, researchers performed a genome-wide association study using DNA collected from individuals with or without UC who shared a similar ancestry, in order to minimize other genetic differences. With this method, they were able to identify chromosomal regions, as well as genes within some of those regions, that are associated with an increased risk of developing UC. More information regarding this study can be found in the Digestive Diseases and Nutrition chapter.

Researchers have identified genetic variations in multiple regions of the genome that are associated with common measures of obesity that reflect total body fat and fat distribution. NIDDK-supported scientists carried out large-scale analyses of data from multiple genome-wide association studies. From this research, they uncovered genetic variations in six previously unreported regions (loci) of the genome that are associated with adult body mass index (a measure of weight adjusted to height). Read more about these findings in the Obesity chapter.

Under circumstances that are not yet completely understood, gout arises when excess uric acid begins to crystallize, causing inflammation and pain in the joints. Seeking to identify genes that contribute to increased uric acid levels and gout, researchers conducted genome-wide association studies. The Kidney, Urologic, and Hematologic Diseases chapter has information on the two new genes that were found to be associated with gout.

Available data suggest that there is a significant genetic predisposition to primary biliary cirrhosis—a chronic disease that causes the bile ducts in the liver to become inflamed and damaged and, ultimately, disappear. Genome-wide association studies were conducted to identify genetic loci associated with increased risk for this disease. The analysis identified several genetic variations in three specific genomic loci that are strongly associated with the patient group. To read more about the recently identified loci, see the Digestive Diseases and Nutrition chapter.

To discover gene variants associated with chronic kidney disease and kidney function, scientists performed genome-wide association scans on nearly 20,000 samples that had been collected during previous trials, and then replicated these results with a separate set of over 21,000 additional specimens. Several loci were found to be associated with kidney function, and one locus was linked to chronic kidney disease. More information on this study can be found in the Kidney, Urologic, and Hematologic Diseases chapter.

Hepatitis C is one of the major causes of chronic liver disease in the U.S. and a common cause of liver cancer. To identify host genetic factors that are required for successful hepatitis C infection, researchers utilized genome-wide scan technology. This approach confirmed the role of genetic factors previously implicated in hepatitis C infection and identified several host factors not previously suspected of playing a role in viral entry into the cell. See the Digestive Diseases and Nutrition chapter for more information.

With exciting genetic findings in hand, scientists can explore how these genes function in health and what goes wrong in disease. Research in this area may also set the stage for even more scientific breakthroughs on other disease-associated factors. For example, a newly associated gene may encode a protein that interacts with numerous other proteins. Therefore, discovering the disease association not only implicates the originally found gene and its encoded protein in the disease, but also the other proteins with which it interacts. This knowledge could illuminate several new therapeutic targets for disease prevention or treatment. Studying genes that were not thought to be involved in a disease can lead to new avenues for research that would likely not have been pursued otherwise. Identifying the functions of genes may not only enhance understanding of molecular mechanisms that underlie disease, but may also reveal new targets for diagnosis, risk assessment, and therapy.

## AUTOIMMUNE DISEASES WITHIN THE NIDDK MISSION

This edition of *NIDDK Recent Advances & Emerging Opportunities* features research efforts that have revealed new information about several autoimmune diseases. Antibodies against one's own proteins

are termed "autoantibodies," and are a hallmark of autoimmune diseases. Scientists are learning more about the targets of the misguided immune attacks, and also are gaining insights into what goes awry in the immune system to unleash self-reactive antibodies. Brief snapshots are presented here, and more complete details of these research advances are found throughout this compendium.

Scientists discovered that in patients with autoimmune diseases such as type 1 diabetes or lupus, an immune system process called "receptor editing" is impaired, leading to the release of immune B cells that aberrantly produce autoantibodies. The immune system has several strategies for preventing the circulation of such destructive B cells throughout the body, one of which is "receptor editing," in which the DNA in the B cell is rearranged or shuffled to prevent the production of "self" antibodies. The finding that this process is impaired in patients with or at risk for autoimmune diseases could facilitate personalized treatments for these patients by helping to determine who might benefit from B cell therapies. For more information on this exciting research advance, see the write-up in the Diabetes, Endocrinology, and Metabolic Diseases chapter.

While studying the development of type 1 diabetes in an animal model, investigators identified a gene that may play a protective role in eliminating immune system cells that can destroy insulin-producing cells. The gene, called *Deaf1*, controls the production of molecules needed to eliminate self-reactive T cells, which are types of immune system cells. Cells in the pancreatic lymph nodes of mice make two forms of Deaf1 protein: a full-length, functional form and a shorter, variant form. Higher levels of the variant form were found to be associated with type 1 diabetes in mice and humans. See the Diabetes, Endocrinology, and Metabolic Diseases chapter for more on this intriguing finding and how it provides a potential target for drug development to treat the disease.

Researchers have recently reported how an immune system protein, called HLA-DQ8, contributes to increased immune reactivity towards dietary gluten in celiac disease. Celiac disease is an immune reaction to gluten, a protein found in wheat, rye, and barley. When people with celiac disease eat foods containing gluten, their immune system responds by damaging the small intestine—an autoimmune destructive condition. HLA proteins are found on the outer portion of cells, and the immune system uses this set of proteins to distinguish "self" cells from invaders. In people with celiac disease, HLA-DQ8 recognizes gluten fragments and presents them to the immune system, which results in a misguided inflammatory response. As described in the Digestive Diseases and Nutrition chapter, researchers show for the first time how the immune system recognizes different forms of gluten to generate an amplified immune response. Delineation of how the initial response develops paves the way for further investigations into how this response contributes to disease onset and progression.

New findings have recently been reported on the immunological events relevant to a debilitating autoimmune disease known as Goodpasture's syndrome (GPS). GPS is a rare condition marked by kidney damage, sometimes leading to kidney failure, and bleeding in the lungs. The underlying cause of GPS results from the body's own production of antibodies against a portion of the type IV collagen protein. As described in the Kidney, Urologic, and Hematologic Diseases chapter, investigators are beginning to uncover how immune B cells may generate antibodies to the body's own type IV collagen.

Researchers have identified a protein that may play a key role in the development of an autoimmune form of kidney disease known as "idiopathic membranous nephropathy." In this study, 70 percent of the tested blood samples from patients with idiopathic membranous nephropathy contained autoantibodies that attacked a single kidney protein; this kidney protein was ultimately identified as the M-type phospholipase $A_2$ receptor, or $PLA_2R$. The identification of the protein that induces the immune response will open new avenues of exploration in idiopathic membranous nephropathy. For additional information on this research advance, see the Kidney, Urologic, and Hematologic Diseases chapter.

## PROTEIN FOLDING AND ENDOPLASMIC RETICULUM STRESS IN DISEASE

The correct three-dimensional structure of a protein is critical to its proper function. "Protein folding"—the

process by which a protein acquires its mature structure—occurs at several locations within the cell, including in the endoplasmic reticulum (ER). The ER performs several other functions, including additional processing of proteins, for example, to attach other necessary molecular components, and transporting proteins to the correct locations for them to perform their respective functions, whether within or outside the cell. These critical processes make the ER an important functional component of the healthy cell. Stress on the ER, whether caused by protein misfolding or other various stimuli, can lead to detrimental consequences, including cell death.

Protein misfolding can occur as a result of gene variations affecting the sequence of amino acids in a protein, or from defects in the folding process. Normally, misfolded proteins are eliminated by a cell's "quality control" system. However, in some cases, these misfolded proteins cannot be eliminated. Many diseases are associated with the aggregation of misfolded proteins, which can block cell function and induce cell death. Diseases also can result from the lack of properly folded proteins—without proper processing of these proteins, they are unable to function effectively. A number of diseases within the NIDDK's mission are known or suspected to be associated with protein misfolding and ER stress, including cystic fibrosis, alpha-1-anti-trypsin deficiency, polycystic kidney disease, type 2 diabetes, and possibly type 1 diabetes and inflammatory bowel disease.

To share information deriving from the study of diverse diseases and spur new research in this field, the NIDDK sponsored a workshop in January 2009 entitled *Protein Misfolding and Misprocessing in Disease*. Sessions of the workshop focused on the basic biology of protein folding and processing, insights gained from research on model organisms, as well as advances in understanding the biology of specific protein misfolding diseases, such as alpha-1-anti-trypsin deficiency, Wolfram syndrome, cystic fibrosis, and congenital hyperinsulinism. Other sessions focused the translation of these basic findings into novel therapeutic strategies, including the development and use of small molecule screens and other tools to identify potential therapeutics for treating a wide variety of diseases associated with protein misfolding.

In a recent advance highlighted in this compendium, scientists identified a factor that links ER stress with a condition called "lipotoxicity," which occurs when the amount of lipid exceeds the storage capacity of fat tissue, as is seen in obese patients. The identification of this factor helps explain how excess lipids can lead to cellular dysfunction, cell death, and organ failure, and may serve as a potential target for therapies directed toward combating obesity-related diseases.

In another advance described in this compendium, scientists discovered that chronic ER stress induced by a high-fat diet and obesity can lead to inappropriate glucose production in the liver. They found that the cell's response to ER stress and production of glucose during fasting shared a protein. When the cells are chronically stressed, this protein is stuck in one position, promoting the release of glucose, and possibly leading to insulin resistance.

Scientists also revealed that amyloids, highly packed protein structures commonly associated with diseases like Alzheimer's and type 2 diabetes, are not always the result of protein misfolding, as previously thought. Rather than a sign of dysfunction, these structures may, in some circumstances, serve an important role in storing hormones in a cell. However, in conditions such as a high-fat diet, stress, or older age, these structures may aggregate, harming the cells where they collect. This build-up of amyloids could explain, in part, the impaired function and loss of insulin-producing beta cells seen in type 2 diabetes.

Finally, scientists in NIDDK's Division of Intramural Research made ground-breaking technical advances to allow them to record how individual protein molecules fold into their correct biological shapes (see later in this chapter).

These exciting advances highlight important progress in understanding one of the biggest research questions in basic biology—protein folding and its role in health and disease. By studying the structural changes associated with going from an unfolded to a folded protein, scientists may discover new insights into the fundamental processes that guide protein folding and identify misfolding steps that can lead to disease. The NIDDK will continue to build on these discoveries and foster research to understand, prevent, and treat diseases

and conditions associated with protein misfolding and ER stress.

## STUDIES IN PROTEIN FOLDING

**Watching Proteins Take Shape One Molecule at a Time:** Scientists in NIDDK's Division of Intramural Research have made ground breaking technical advances that allow them to study how individual protein molecules "fold" into their correct biological shapes. When a protein is made in a cell, it folds into a uniquely defined three-dimensional structure. This "native" structure, as it is called, is intimately linked to the protein's function, allowing the protein to properly perform chemical reactions, turn genes on or off, or interact with other proteins to regulate cellular communication. In cases where a protein's native structure is disrupted, such as by genetic mutations that alter its ability to fold properly, the resulting impairment of the protein's normal function can cause disease.

Given the importance of protein structure and function in health and disease, scientists have been interested in understanding the molecular determinants that lead to the formation of a protein's correct native structure. Traditional biophysical techniques have been used to uncover considerable knowledge about the structures of proteins in native and unfolded states and the factors that determine whether a protein folds or not. These techniques, however, have not allowed researchers to visualize the actual "path" or series of molecular events that takes a protein from the unfolded state to the native state. In a technical tour-de-force, NIDDK scientists have pushed the limits of a technique—called single-molecule fluorescence resonance energy transfer—to view, for the first time, the transitions between unfolded and native states during the folding process of individual protein molecules. By overcoming a number of technical issues that limited this technique, they were not only able to record discrete transitions between the unfolded and native states, but were also able to define an upper limit on the amount of time it takes for these transitions to occur. In addition to the study's technical achievement, the ability to observe individual folding transitions has important implications for the further study of protein folding. As all of the important structural changes associated with going from the unfolded state to the native state occur during these transitions, the application of this technique may allow researchers to uncover the fundamental mechanisms that guide the protein folding process and identify the misfolding steps that are often associated with diseases.

*Chung HS, Louis JM, and Eaton WA: Experimental determination of upper bound for transition path times in protein folding from single-molecule photon-by-photon trajectories. Proc Natl Acad Sci USA 106: 11837-11844, 2009.*

## DELIVERING ANTI-INFLAMMATORY THERAPY

**New Potential Therapeutic Strategy To Suppress Inflammation:** Scientists have developed new technology with potential as a therapeutic strategy for inflammatory diseases by blocking production of proteins mediating inflammation. Inflammation is a key contributor to a variety of disorders including insulin resistance, cardiovascular disease, and autoimmune diseases. Short molecules of ribonucleic acid (RNA) can be targeted to reduce levels of specific proteins by interacting—or interfering—with the genetic material that encodes the protein, to prevent the protein from being made. This technique, known as "RNA interference," has potentially transformative therapeutic value. The development of a safe and effective way to deliver short interfering RNA molecules (siRNA) to specific types of cells *in vivo*, however, would be required before this technique could be used therapeutically. In a recent study, scientists designed a method to orally deliver siRNA to mouse macrophages, a cell type of the immune system that is important in initiating the inflammatory response. In this novel approach, layers of RNA molecules can be encapsulated within hollow, porous, tiny (micron-sized) shells of a substance called beta1,3-D-glucan, a non-toxic material made by yeast cells. The shells are recognized by proteins found primarily on the surface of macrophages, allowing for the specific uptake of the shells by macrophages. The scientists termed these shell particles "GeRPs" or glucan-encapsulated siRNA particles.

To test this system as a potential therapeutic in animals, the investigators examined the effects of feeding mice GeRPs with siRNA to a specific inflammatory protein known as Map4k4. They detected GeRPs inside

mouse macrophages in various tissues of the mouse body, including spleen, liver, and lungs, and observed a decrease in levels of Map4k4 in these tissues. The mice fed GeRPs with siRNA to Map4k4 were then given a toxic chemical that mimics a bacterial infection in order to stimulate the inflammatory response. When mice without the siRNA were given the chemical, their macrophages stimulated an excessive inflammatory response that was fatal to the animals. By feeding the mice siRNA to Map4k4, the scientists were able to halt the inflammatory response to the chemical, thus protecting the mice. This exciting result demonstrated that the orally administered siRNA was not only delivered to the correct cells—the macrophages—and carried to multiple tissues, but that the siRNA also reduced levels of Map4k4 and thus altered the mouse inflammatory response.

Inflammatory responses triggered by macrophages are involved in many conditions, including obesity, type 2 diabetes, inflammatory bowel disease, colitis, cardiovascular disease, atherosclerosis, and rheumatoid arthritis. The GeRP technology provides a novel oral delivery system for RNA interference to reduce levels of proteins involved in inflammation. Although further development and testing of the GeRP delivery system in animal models and in humans will be required, this study reveals the exciting potential of a new therapeutic strategy to suppress inflammation that may be applied to numerous adverse health conditions. In addition, this technology could be used to deliver siRNA, and possibly other cargo, to other types of cells in the immune system to alter their function and therefore could be explored as a potential therapy for autoimmune diseases like type 1 diabetes.

*Aouadi M, Tesz GJ, Nicoloro SM, Wang M, Chouinard M, Soto E, Ostroff GR, and Czech MP: Orally delivered siRNA targeting macrophage Map4k4 suppresses systemic inflammation.* Nature *458: 1180-1184, 2009.*